Plant Division: Remembering Where to Build the Wall

Before mitosis, a band of microtubules accurately forecasts where the next cross-wall will be inserted but then depolymerizes. How is this division plane memorized until cytokinesis? The molecular memory is being uncovered.

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In plants, cellular space is mapped out according to the orientation of new cross-walls. Cell expansion may subsequently distort this pattern, but the absence of cell migration means that the spatial relationships between cells are very largely determined at cytokinesis. Notably, these division planes in plants are not negotiated during division but in the run-up to mitosis. Over forty years ago it was discovered that a cortical band of microtubules, which forms in preprophase, anticipates where the dividing wall will attach to the parental wall in the final stage of cytokinesis [1,2]. However, once the cell enters mitosis, this preprophase band of microtubules disassembles, leading to the question of how the prepared site is memorized until cytokinesis. In a recent issue of *Current Biology*, Walker *et al.* [3] have now revealed that the preprophase band leaves behind a molecular tidemark in the form of a ring of protein.

During morphogenesis, the preprophase band forecasts all kinds of division — curved, straight, asymmetric — in somatic plant cells [4]. This band is initially broad and forms at the cortex amongst the more evenly distributed interphase microtubules. It does not constrict into the cell, as in the contractile division of animal cells, but narrows upon the cortex. So what exactly does the band predict? During the latter stages of mitosis, the cytokinetic apparatus — the phragmoplast — evolves out of the central spindle [5]. In animal cells this spindle residue becomes the midbody but, in plant cells, vesicles are brought to the midline of this structure where they fuse to form a small central disk known as the cell plate [6]. The ring of phragmoplast microtubules at the leading edge of the growing cell plate becomes increasingly wider until it contacts the parental wall precisely at the site formerly occupied by the preprophase band. Not only does the fusion of vesicles contribute membrane to the new cross-wall but their contents also provide the polysaccharide callose. This flexible material supports the growing cell plate, but, after the plate has attached to the parental wall, it is replaced by the inelastic cellulose microfibrils of the cell wall proper.

In large cells, the cytokinetic disk has to grow long distances across the vacuole, highlighting the question of what guides the leading edge of the phragmoplast/cell plate on its journey to the cortex. Cell plates developing from spindles displaced by centrifugation curve back towards the cell's equator [7] and it is reasonable to think that a cytoskeletal component provides the 'pulling force' that reconnects the cell plate to the specialized site. That the dividing nucleus is physically connected to the cortex was discovered by light microscopy some twenty years before the preprophase band. In mature cells induced to divide, the nucleus migrates into the centre of the large vacuole on cytoplasmic strands that initially form a three-dimensional star [8]. These strands then coalesce into a two-dimensional transvacuolar sheet — the phragmosome — within which the nucleus divides. Given that the cell plate then expands within this cytoplasmic raft, the phragmosome inevitably represents the division plane and is known to have the preprophase band at its perimeter [9]. One suggestion is that the narrowing of the cortical microtubules into the band functions to draw into the division plane the cytoplasmic strands radiating from the nucleus [10]. Hence the suspension of the phragmosome across vacuolated cells makes it clear that there is a physical pathway connecting the central nucleus to a specific zone of the cortex. But what makes that zone different from the rest of the cortex?

In addition to microtubules, the radial strands connecting the nucleus and cortex contain actin filaments and they invade the preprophase band to provide another premitotic marker. However, this preprophase band of actin depolymerizes along with the microtubules at prometaphase. Actin persists in the phragmosome as well as other parts of the cortex but its specific exclusion from the band now creates an actin-depleted zone [11]. Actin therefore acts as a positive marker of the future division plane in preprophase, then as a negative marker of this plane within the cortex in mitosis [12]. The plant-specific kinesin, KCA1, which localizes to the plasma membrane, also becomes depleted from the cortical band throughout mitosis [13]. Although another plant-specific protein TPLATE is not involved in the premitotic establishment of the division site, it does provide a positive marker in the closing stages of cytokinesis. The protein, which localizes to the cell plate from early phragmoplast formation, spreads within the cortical division site when the cell plate makes contact [14]. The suggestion is that TPLATE is important for vesicle trafficking and correct insertion of the cell plate. The microtubule-associated protein, AIR9, is interesting in that it marks the division site in preprophase, disappears during mitosis, reappears as a ring just as the phragmoplast makes contact, then migrates into the plate [15,16]. Contact of ectopic cell plates outside the former preprophase band zone does not induce the reappearance of the AIR9 ring, suggesting that its return requires contact with another component of the band's molecular memory.

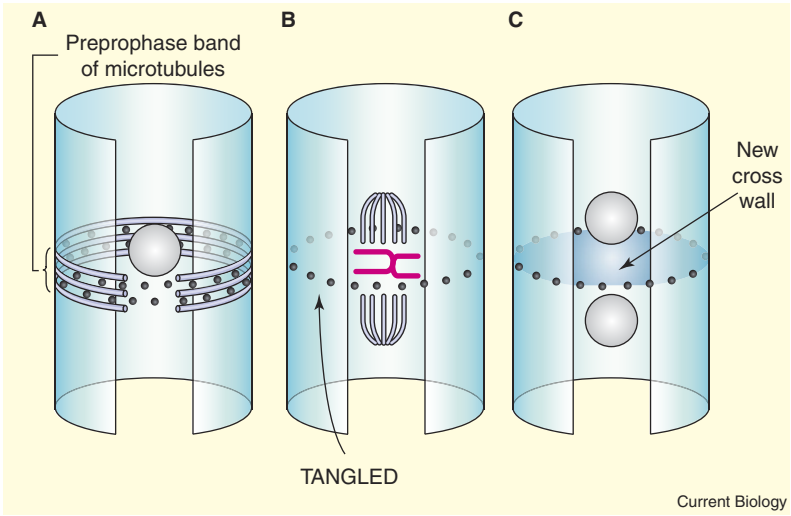

Figure 1. TANGLED remembers the predetermined site of the division plane.

(A) Microtubules aggregate to form a preprophase band that predicts the division plane. This draws TANGLED into the cortical division site. (B) During mitosis the cortical microtubules depolymerize, leaving TANGLED to memorize the cortical division site. (C) In cytokinesis, the new cross wall (the cell plate) expands outwards from the centre of the cell until it attaches to the parental cell wall at the cortical site forecast by the ring of TANGLED protein during preprophase.

Ectopic plates failing to exhibit the AIR9 signal retain the 'immature' wall component callose for longer than normal, pointing to a role for AIR9 in cross-wall maturation.

Analyses of mutants has provided important support for the role of the preprophase band in establishing the division site. *ton1* and *ton2/fass* mutants [17,18] of *Arabidopsis* are short and squat with disturbed tissue organization. Mutant cells do not form preprophase bands and as a result cross-walls are inserted in a random fashion. The maize mutant, *tangled*, causes leaf cells to divide in abnormal orientations with longitudinal divisions becoming crooked or curved. Some of the preprophase bands are occasionally misoriented and the outgrowth of phragmoplasts misguided [19]. The phragmoplast-orienting kinesins POK1 and POK2 interact with maize TANGLED in yeast two-hybrid assays and the *Arabidopsis pok1;pok2* double mutant shows a phenotype very similar to maize *tangled* [20]. The recent study by Walker *et al.* [3] reports that TANGLED is the first protein known to remember, without interruption, the predetermined site of division throughout mitosis (Figure 1). In support of this conclusion, microtubules are shown to bring *Arabidopsis* TANGLED to the division site before mitosis but, when the preprophase band depolymerizes in mitosis, the protein remains as a thin punctate ring, only disappearing upon completion of cytokinesis. A persistent ring of TANGLED tends not to form in the *pok1;pok2* double mutants, demonstrating the importance of these kinesins in delivering TANGLED to the division site. Not all *Arabidopsis tangled* cells show abnormal wall patterns so, assuming that the mutations are completely null, *Arabidopsis* TANGLED can only be part of the mechanism for division plane determination. The search now continues for other components of the division ring and insights into the attractive influence they exert over the leading edge of the cytokinetic apparatus.

References

1. Pickett-Heaps, J.D., and Northcote, D.H. (1966). Organization of microtubules and endoplasmatic reticulum during mitosis and cytokinesis in wheat meristems. J. Cell Sci. *1*, 109–120.
2. Pickett-Heaps, J.D., and Northcote, D.H. (1966). Cell division in the formation of the stomatal complex of young leaves of wheat. J. Cell Sci. *1*, 121–128.
3. Walker, K.L., Muller, S., Moss, D., Ehrhardt, D.W., and Smith, L.G. (2007). *Arabidopsis* TANGLED identifies the division plane throughout mitosis and cytokinesis. Curr. Biol. *17*, 1827–1836.
4. Gunning, B.E.S., Hardham, A.R., and Hughes, J.E. (1978). Pre-prophase bands of microtubules in all categories of formative and proliferative cell division in Azolla roots. Planta *143*, 145–160.
5. Lloyd, C., and Chan, J. (2006). Not so divided: the common basis of plant and animal cell division. Nat. Rev. Mol. Cell Biol. *7*, 147–152.
6. Samuels, A.L., Giddings, T.H. Jr., and Staehelin, L.A. (1995). Cytokinesis in tobacco BY-2 and root tip cells: a new model of cell plate formation in higher plants. J. Cell Biol. *130*, 1345–1357.
7. Ôta, T. (1961). The role of cytoplasm in cytokinesis of plant cells. Cytologia *26*, 428–447.
8. Sinnott, E.W., and Bloch, R. (1940). Cytoplasmic behavior during division of vacuolate plant cells. Proc. Natl. Acad. Sci. USA *26*, 223–227.
9. Venverloo, C.J., Hovenkamp, P.H., Weeda, A.J., and Libbenga, K.R. (1980). Cell-division in Nautilocalyx explants. 1. Phragmosome, preprophase band and plane of division. Z. Pflanzenphysiol. *100*, 161–174.
10. Flanders, D.J., Rawlins, D.J., Shaw, P.J., and Lloyd, C.W. (1990). Nucleus-associated microtubules help determine the division plane of plant epidermal cells: avoidance of four-way junctions and the role of cell geometry. J. Cell Biol. *110*, 1111–1122.
11. Cleary, A. (1995). F-actin redistributions at the division site in living Tradescantia stomatal complexes as revealed by microinjection of rhodamine-phalloidin. Protoplasma *185*, 152–165.
12. Hoshino, H., Yoneda, A., Kumagai, F., and Hasezawa, S. (2003). Roles of actin-depleted zone and preprophase band in determining the division site of higher-plant cells, a tobacco BY-2 cell line expressing GFP-tubulin. Protoplasma *222*, 157–165.
13. Vanstraelen, M., Van Damme, D., De Rycke, R., Mylle, E., Inze, D., and Geelen, D. (2006). Cell cycle-dependent targeting of a kinesin at the plasma membrane demarcates the division site in plant cells. Curr. Biol. *16*, 308–314.
14. Van Damme, D., Coutuer, S., De Rycke, R., Bouget, F.Y., Inze, D., and Geelen, D. (2006). Somatic cytokinesis and pollen maturation in Arabidopsis depend on TPLATE, which has domains similar to coat proteins. Plant Cell *18*, 3502–3518.
15. Buschmann, H., Chan, J., Sanchez-Pulido, L., Andrade-Navarro, M.A., Doonan, J.H., and Lloyd, C.W. (2006). Microtubule-associated AIR9 recognizes the cortical division site at preprophase and cell-plate insertion. Curr. Biol. *16*, 1938–1943.
16. Buschmann, H., Sanchez-Pulido, L., Andrade-Navarro, M.A., and Lloyd, C.W. (2007). Homologues of *Arabidopsis* microtubule-associated AIR9 in trypanosomatid parasites: Hints on evolution and function. Plant Signal Behav. *2*, 296–299.
17. Torres-Ruiz, R.A., and Jurgens, G. (1994). Mutations in the FASS gene uncouple pattern formation and morphogenesis in Arabidopsis development. Development *120*, 2967–2978.
18. Traas, J., Bellini, C., Nacry, P., Kronenberger, J., Bouchez, D., and Caboche, M. (1995). Normal differentiation patterns in plants lacking

microtubular preprophase bands. Nature *375*, 676–677.
19. Cleary, A.L., and Smith, L.G. (1998). The Tangled1 gene is required for spatial control of cytoskeletal arrays associated with cell division during maize leaf development. Plant Cell *10*, 1875–1888.
20. Muller, S., Han, S., and Smith, L.G. (2006). Two kinesins are involved in the spatial control of cytokinesis in Arabidopsis thaliana. Curr. Biol. *16*, 888–894.

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Visual Cortex: More Wiggle Room for the Brain

Experiments in which one eye of a ferret is removed at birth show subtle effects on the development of visual cortex maps that are in agreement with those predicted by theory.

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The philosopher John Locke was the first to argue that knowledge of the world can only be acquired empirically, through the operation of our sense organs. He proposed that, at birth, the human mind (and by implication the cerebral cortex) was a *tabula rasa* lacking information about the properties of the natural world. No one now accepts Locke's proposition completely, given the evidence, for example, that newborn babies can respond preferentially to faces, which implies that genes somehow contain information about faces and can translate it into appropriate kinds of neural connectivity. But questions concerning the influence of very early sensory experience and the extent to which brain structures are, or are not, genetically programmed continue to be of central importance to research on cortical development. The issues come into particularly clear focus in studies of visual cortex. Although the mechanisms of early cortical development are arguably best understood in this part of the brain, knowledge of what goes on in the time between the first migrations of neurons along radial glia to form cortical layers *in utero*, and the emergence of a functionally mature visual system some time after birth, is still very limited. Numerous studies have so far failed to determine whether basic aspects of visual cortex organization — neuronal receptive field properties and columns and maps — are determined by cues directly controlled by patterns of genetic expression, or whether development is a flexible, self-organizing process more likely to be influenced by neural activity and patterns of sensory stimulation. A recent study by Farley *et al.* [1] lends support to the latter proposition although, in my view, it does not unambiguously settle the debate.

Wiesel and Hubel [2] provided the first evidence that the early development of visual cortex could be altered by a change in sensory stimulation. In a classic experiment they closed one eye of a newborn kitten or a monkey. Ocular dominance columns — roughly half-millimetre wide regions of cortex running perpendicularly from *pia* to white matter (hence the term 'column'), containing cells that respond preferentially to one or the other eye — changed size [3]. Those connected to the seeing eye became wider and took over cortical territory made available by the shrinkage of columns connected to the closed eye. While this showed that altered visual experience could change the outcome of early visual development, it did not show whether visual experience was actively involved in setting up the columns to begin with. In fact, subsequent observations by Wiesel and Hubel [4] and others showed that visual experience does not play an active role in setting up columns. Thus, normal-looking ocular dominance columns and orientation columns — columns of cells having the same preference for stimulation

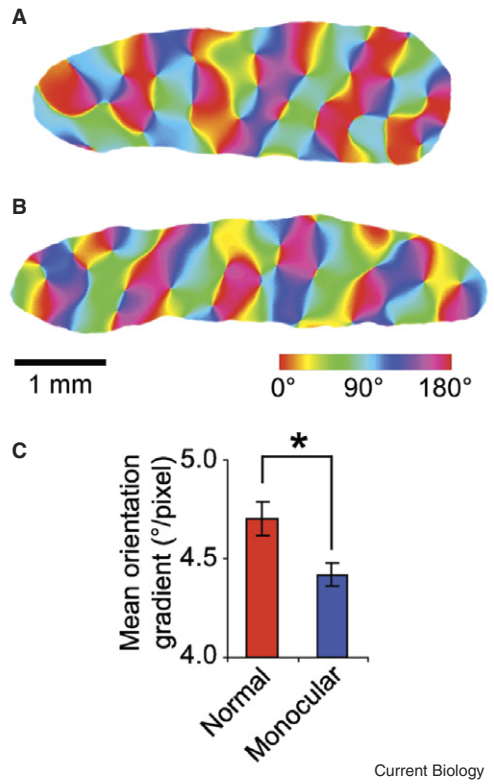

Figure 1. Perturbing orientation map development.
(A) Orientation map from a normal ferret with pixels colour-coded according to the orientation preferences of neurons at each location. (B) Orientation map from a ferret enucleated at birth. Differences in the periodicity of the patterns in (A) and (B) are not visually obvious but can be detected by Fourier analysis. (C) Mean orientation gradient values for normal (n = 10) and enucleated (monocular) (n = 12) ferrets. Error bars show the S.E.M. Gradient values are lower in the monocular ferrets, consistent with a lower spatial periodicity. (Adapted from [1].)